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Heavy Breathing: Energy Conversion by Mitochondrial Respiratory Supercomplexes

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The phrase "respiratory chain" implies that energy that is ultimately derived from mitochondrial oxidative phosphorylation is produced via a linear arrangement of discrete electron transfer complexes. A recent paper in *Molecular Cell* (Acin-Pérez et al., 2008) calls this model into question.

In 1926, David Keilin published a seminal paper describing the role of hemoproteins, called cytochromes, in the "horizontal" transfer of electrons derived from cellular dehydrogenases to an oxygen-activating oxidase, all located in the mitochondrial inner membrane (MIM). Otto Warburg would call it the respiratory chain (Atmungskette).

Electrons → cytochrome *b* →

cytochrome *c* → cytochrome *a* →

cytochrome *a*3 → oxygen

In the early 1960s, Peter Mitchell showed that besides this horizontal flow of electrons, there is a concomitant vectorial transport of dehydrogenase-derived protons "vertically" across the MIM (from the matrix to the intermembrane space). This creates a proton gradient across the MIM that, together with its electrical component, can be used to drive ATP synthesis by the F_oF_1-ATP synthase, a remarkable machine that uses both gradients to drive a rotary motor to convert ADP to ATP.

In the ensuing years, this picture of the respiratory chain/oxidative phosphorylation system (OXPHOS) has been fleshed out by the description of the four major protein complexes that facilitate this flow of electrons and protons. We now know that both complexes I (an NADH dehydrogenase) and II (a succinate dehydrogenase) transfer electrons from TCA cycle-generated NADH and $FADH_2$ to ubiquinone (also called coenzyme Q), then to complex III (ubiquinone oxidoreductase), then to cytochrome *c*, then to complex IV (cytochrome *c* oxidase), and finally to molecular oxygen, ultimately producing water. At the same time, protons cross the inner membrane at complexes I, III, and IV to generate the electrochemical proton gradient that will be used by the ATP synthase (called complex V, but technically not a member of the respiratory chain).

Since the consolidation of this view of the OXPHOS system, one contentious issue has been the nature of its physical organization. Two alternative models have been proposed (Figure 1). The currently favored textbook view is the "random collision" model (Hackenbrock et al., 1986): all components of the respiratory chain diffuse individually in the membrane, and electron transfer depends on the random, transient encounter of the four individual protein complexes and the two smaller mobile electron carriers, CoQ and cytochrome *c*; ATP synthase is assumed to diffuse laterally in the membrane as an individual entity and/or as homo-oligomers. In the "solid-state" model, proposed more than 50 years ago (Chance and Williams, 1955), the substrate is channeled directly from one enzyme to the next, with the enzyme components assembled into a huge supramolecular energy-converting machine. In 2000, Helmut Schägger's group, using western blotting of megadalton-sized respiratory complexes separated by "blue native gel electrophoresis" (BNGE), showed that I-III-IV supercomplexes could indeed be isolated from mammalian cells, and III-IV supercomplexes from baker's yeast (which lack an OXPHOS-linked complex I) (Schägger and Pfeiffer, 2000). Such an arrangement would likely increase the efficiency of electron/proton flow and hence that of ATP synthesis while also minimizing the generation of potentially toxic reactive oxygen species that have been proposed to be involved in the pathogenesis of neurodegenerative diseases.

Kinetic evidence from inhibitor-titration studies of isolated mitochondria is consistent with both the random collision and solid-state models, suggesting the coexistence of individual complexes and supercomplexes (Bianchi et al., 2004). However, rather than resolving the issue of how the respiratory chain is organized topographically, controversy has ensued, focused mainly on the technicalities of BNGE methodology—especially whether supercomplexes observed by BNGE are nothing more than "aggresomes" of highly hydrophobic individual complexes stuck together during isolation.

In the November 21 issue of *Molecular Cell*, Acin-Pérez et al. (2008) weigh in on the side of supercomplexes. In so doing, they help resolve some questions but raise new ones. In this paper, BNGE is still the method of choice, but three key elements have been added. First, supercomplexes, or portions thereof, were found to be absent upon analysis of respiratory chain complexes from cells containing mutations in proteins required for the structure, function, and assembly of complexes I, III, or IV. Second, pulse-chase experiments showed convincingly that there is a temporal gap between the formation of the individual complexes and that of the supercomplexes. Both approaches undercut the argument that supercomplexes are mere BNGE aggregation artifacts. Finally, in a compelling technical tour de force, Acin-Pérez et al. were able to isolate minuscule amounts of supercomplex assemblies and subassemblies directly from the BNGE gels and show, using respirometry, that these

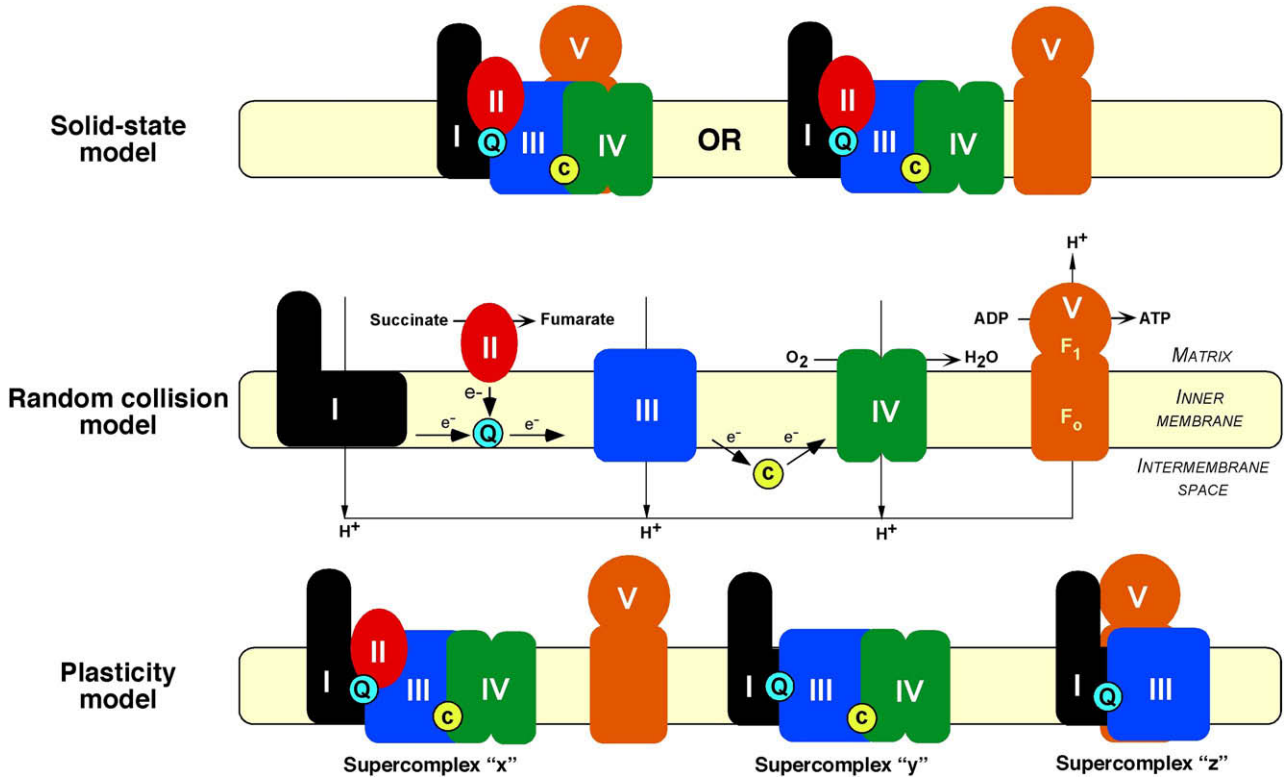

Figure 1. Comparison of Models for the Organization of OXPHOS Complexes
See text for details. For simplicity, all components are shown as monomers, but the reality is more complicated. For example, complex III exists as dimers, and complex V as monomers, dimers, or homo-oligomers, and supercomplexes contain between 1 and 4 complex IV's. F_o and F_1 refer to the ATP synthase subassemblies located in the inner membrane and matrix, respectively. I–V, complexes I–V; Q, coenzyme Q; c, cytochrome c; H^+, protons, e^-, electrons.

assemblies could transfer electrons from NADH to O_2—that is, they could respire! Moreover, the supercomplexes were respiratorily incompetent following treatment with classic inhibitors, such as rotenone, antimycin, and cyanide (affecting complexes I, III, and IV, respectively). Accordingly, the authors deem these supramolecular structures to be not only supercomplexes but authentic "respirasomes."

Interestingly, the supercomplexes reported by Acin-Pérez et al. contain not only complexes I, III, and IV, as had been observed previously (Schäfer et al., 2007), but also, for the first time, complex II. As such, it would have been helpful if a complex II inhibitor, such as 3-nitropropionic acid, had also been used in the respirometry assays. Since different supercomplex types were observed (e.g., I-II-III-IV, I-III-IV, and I-III-V), the authors suggest that the solid-state model should be modified to accommodate the coexistence of both individual complexes (as in the random collision model) and various supercomplex subtypes, whose composition may vary according to tissue type or to the temporal exigencies of bioenergetic demand (a "plasticity" model).

The finding by Acin-Pérez et al. of complex V in supercomplexes is also intriguing. If the ATP synthase functions as part of the supercomplex (as opposed to a separate "ATP synthasome" composed of ATP synthase with the carriers for phosphate and ADP/ATP; Chen et al., 2004), it must do so in a way that allows for rotary catalysis to occur, and as a dimer no less. Since the authors only used antibodies to the membrane-embedded F_o subcomplex of ATP synthase, and not to any of the catalytic F_1 subunits, it is formally possible that the supercomplexes contain only F_o subunits (perhaps to facilitate cristae formation; Paumard et al., 2002), while functional ATP synthases are present as individual monomeric and homo-oligomeric complexes. Since dimers of ATP synthase are similar in size to supercomplex(I_1-III_2), it is formally possible that its presence in the supercomplex is a BNGE artifact. More compelling proof that ATP synthase is truly a component of the supercomplex, such as an antibody-shift experiment, may be required.

The solid-state and/or plasticity models would help explain the puzzling observation that mutations in, for example, complex I can sometimes cause combined complex I+III deficiency in some patients with mitochondrial disease (Budde et al., 2000). It should be noted, however, that most pathogenic mutations in respiratory chain proteins result in isolated deficiency of the relevant complex, with no such "crosstalk" among complexes. On the other hand, cardiolipin has been shown to be required for supercomplex assembly, and mutations in tafazzin, a protein required for cardiolipin synthesis, cause Barth syndrome, a mitochondrial disorder that has now been associated both with supercomplex assembly and with deficiency of complexes I, III, and IV (but, interestingly, not II) (McKenzie et al., 2006).

It is now clear that supercomplexes indeed exist and can function as respirasomes. As is usually the case, this finding raises new questions regarding the potentially dynamic nature of supercomplex

architecture, the nature of the association of the ATP synthase with the respirasome, and the role of supercomplexes in human mitochondrial disease and in aging. Sorting out these issues should be interesting, but technically challenging. Given this context, you might not want to hold your breath waiting for the answers.

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Models Use Leptin and Calculus to Count Calories

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Thermostats have "set-points" that engineers design with mathematical rigor. Work in this issue of *Cell Metabolism* (Tam et al., 2009) applies similar modeling strategies to explore the control of murine energy and body weight homeostasis by leptin.

Despite their best efforts and intentions, many people who are overweight or obese find it difficult to lose weight and then maintain their weight loss. This observation, combined with recent basic mechanistic discoveries, suggest the existence of a body weight "set-point"—an idea borrowed from the field of engineering where feedback control systems are designed to regulate a particular variable to match a specified target. In the case of body weight regulation, arguments about the applicability of the set-point concept have been ongoing for decades (Cabanac, 2001; Hervey, 1969; Mrosovsky and Powley, 1977; Reddingius, 1980; Wirtshafter and Davis, 1977). But much of this debate occurred prior to the discovery of leptin, which we now know acts as an adiposity feedback signal to the hypothalamic control system (Zhang et al., 1994). Most researchers believe that this leptin feedback system plays a predominant role in the regulation of body weight, but the kind of feedback control strategy underlying this system remains unclear. A new study in this issue uses mathematical modeling to examine this question (Tam et al., 2009).

To investigate how a system behaves when subjected to different feedback control strategies, engineers commonly develop mathematical models to help understand the behavior of the regulated system. Can a mathematical model of energy metabolism be developed to test various hypotheses about how leptin acts to control body weight? While several groups have begun to develop realistic mathematical models of energy metabolism and body weight change (reviewed in Chow and Hall, 2008), the study by Tam et al. (2009) expands on previous models by explicitly describing the roles of leptin in modulating both food intake and energy expenditure in mice. In particular, Tam et al. used their models to investigate how leptin might act in hypothetical feedback control strategies and determined which control strategy is most consistent with the data.

One strategy for controlling body weight, called proportional feedback control, involves measuring leptin and adjusting food intake or energy expenditure in proportion to the difference between the current leptin level and its set-point value. With proportional control there has to be an "error" to produce a controller output, and therefore a small offset of leptin away from its set-point will always be present. In another control strategy, referred to as integral control, the controller is proportional to both the magnitude and duration of the set-point offset. Often, these two strategies are used in combination in a control strategy called proportional integral control. The integral action eliminates the set-point error remaining when proportional control is used alone.

In their set-point model, Tam et al. implemented a proportional integral control strategy for body weight regulation and